CIGUATERA: TROPICAL FISH POISONING

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Explanatory Note

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CIGUATERA: TROPICAL FISH POISONING

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PART I--HISTORICAL BACKGROUND

Introduction

Ciguatera, poisoning due to the ingestion of the flesh of certain species of fish, has been known for a number of centuries in the subtropical and tropical waters of the Caribbean Sea, the Atlantic Ocean, and Pacific Ocean. The writings of early colonists of the Caribbean Islands and explorers of the Pacific Islands make numerous references to this phenomenon. A review of the literature on Ciguatera reveals that most of the material written previous to the latter part of the 19th century is of historical interest only. The more recent of the investigations have been primarily concerned with reporting the cases, the symptomology of the poisoning, and the species of fish involved. The causative agent or agents responsible for the poisoning have never been demonstrated, except in the case of poisoning by species of puffers (Tetrodon spp.).

Origin of the Term "Ciguatera"

"Ciguatera " is the term popularly used for fish poisoning in the West Indies. The origin of this term has been traced by Poey (1866) to the early Spanish settlers of Cuba, who used it in reference to the digestive and nervous upsets caused by the ingestion of the snail (Turbo pica), commonly known as the Cigua. In time the term was extended to similar digestive and nervous upsets caused by any type of seafood. It is sometimes used in the literature in reference to the frequently fatal poisoning caused by the ingestion of species of puffers (Tetrodon spp.) and porcupine fish (Diodon spp.). However, in this paper it shall be applied more specifically to the nonfatal poisoning caused by the ingestion of other species of fish. For a review of Tetrodon fish poisoning, the reader is referred to Yudkin (1944).

Species of Fish Involved

From time to time numerous species of fish have been reported responsible for poisoning outbreaks. However, not all fish of a given species are toxic. It is known that of fish caught in the same area, at the same time, and of the same species, some will prove to be toxic while others will be nontoxic. No data are available as to the proportion of fish of a so-called "poisonous species" which may be expected to prove toxic. Lists of fish which have caused poisoning or which have been suspected of causing poisoning have been compiled by Poey (1866), Gatewood (1909), Seale (1912), Mowbray (1916), Phisalix (1922), Hoffmann (1927 and 1931), and Gilman (1942).

In the Caribbean area the poisoning outbreaks are in most cases attributable to a fairly small number of species of fish of the families Carangidae, Scombridae, and Sphyraenidae, notably the amberjack (Seriola falcata); the horse-eyed jack (Caranx latus); the king mackerel (Scomberomorus cavalla); and the great barracuda (Sphyraena barracuda). Certain other fish occasionally have also been reported to be poisonous; these are the hogfish (Lachnolaimus maximus); the red snapper (Lutjanus blackfordii); the red grouper (Epinephelus morio); the yellowfin grouper (Mycteroperca venenosa); and the tiger rockfish (Mycteroperca tigris). However, the amberjack, the horse-eyed jack, the king mackerel, and the barracuda are the most consistent offenders.

In the Pacific area, most of the poisoning cases reported have been caused by puffers and porcupine fish. However, the following fish also have been reported toxic; barracuda; Caranx spp.; the red snapper (Lutjanus bohar); a grouper (Epinephelus sp.); the red sea bass (Variola louti); the black sea bass (Serranus fuscoguttatus); and the oilfish (Ruvettus pretiosus).

Table 1 lists some of the fish reported as poisonous in the Caribbean Sea and the Pacific Ocean. It is doubtful whether these fish implicated in the poisoning were accurately identified. This is not surprising inasmuch as few of the investigators were ichthyologists, and many of the fish have different common names in different localities. Identification of the species is often difficult even when a sample of the fish is available, yet it has been found that in some instances, that identification of the species had been determined by ichthyologists from a description given to them by the investigator of the poisoning outbreak who in turn had obtained it from the patient. This merely contributes to the already existing confusion regarding fish poisoning.

Table 1.--Some fish causing poisoning or suspected of having caused poisoning as reported in the literature

Scientific Name	Common Name	Area in which Poisonous
Sphyraena barracuda[1/]	Great barracuda, picuda	Caribbean and Pacific
Scomberomorus regalis	Cero, pintado	Caribbean
Scomberomorus cavalla	King mackerel, sierra	Caribbean
Caranx hippos	Common jack, crevalle	Caribbean
Caranx latus	Horse-eyed jack, jurel	Caribbean
Caranx ruber	Skipjack, cavalla	Caribbean
Caranx crysos	Blue runner	Caribbean
Caranx bartholomaei	Yellow jack	Caribbean
Caranx lugubris	Tinosa	Caribbean and Pacific
Seriola falcata	Amberjack, mandregal	Caribbean
Mycteroperca venenosa	Yellowfin grouper	Caribbean
Mycteroperca tigris	Tiger rockfish	Caribbean
Lachnolaimus maximus	Hogfish	Caribbean
Lutianus blackfordii	Red snapper	Caribbean
Lutianus bohar	Red snapper	Pacific
Ruvettus pretiosus	Oilfish	Pacific
Variola louti	Red sea bass	Pacific
Serranus fuscoguttatus	Black sea bass	Pacific
Epinephelus morio	Red grouper, mero	Caribbean

1/ Barracuda in south seas causing fish poisoning have been identified as S. forsteri and S. picuda

Localities in which Fish Poisoning is Prevalent

It is commonly believed that the poisoning is largely a local occurrence. Certain species of fish are supposed to be toxic only in certain well-defined areas but nontoxic in others. For example, in the Caribbean area, Poey (1866) mentioned that in the banks of the Hormigas, Bahamas Banks, Islandes las Mujeres, and Sanda de Campeche, all fish except the groupers (known locally as cherna) are supposed to be toxic. Rogers (1899) also wrote that the same was true of the banks of the Hormigas and similar areas found near Anegada. Mowbray (1916) pointed out that on Grand Turk Island, which is 6 miles long and $1\frac{1}{2}$ miles wide, fish from the north side are often more toxic than those from the south side. O'Neill (1938) stated that fish are toxic in specific areas near St. Thomas, V. I. (east and south of St. Thomas, Culebrita and the north coast of Vieques Island). Hoffmann (1931) stated that fish of the north coast of Cuba are toxic while those from the south coast are not. Natives of St. Thomas believe that fish taken near Sail Rock are toxic, and that all fish from the vicinity of Peter Island are poisonous. In Puerto Rico, the fishermen claim that the toxic area extends from Salinas Playa on the south to Fajardo on the east. Phisalix (1922) stated that, in the Pacific, entire atolls have been declared "taboo" by natives, and that along the coast of certain islands all fish are toxic. Gatewood (1909) reported that the snapper (Lutjanus bohar)

is always toxic in other areas. Vonfraenkel and Krick (1945) stated that barracuda from the Marshall and Marianas Islands area are poisonous, but those from the Carolina Islands are not. Simmons (1944) listed many areas in the Pacific where toxic fish are found.

The fact that the same species of fish are toxic in one area and not in another is interesting. Numerous explanations have been advanced to explain this phenomenon; unfortunately, none is supported by concrete investigative evidence. Most of the investigators presuppose that the so-called toxic areas contain toxic material on which the fish feed. This toxic material is then absorbed or deposited in the flesh of the fish, rendering it toxic. The fact that these fish are all pelagic makes it difficult to lend credence to this hypothesis. It can be pointed out at this time that very little is known of the migratory behavior of these fish.

Symptoms of Ciguatera

The symptoms associated with Ciguatera in the Caribbean area as described by Mann (1938) and O'Neill (1938) vary slightly from the symptoms of fish poisoning caused by the Pacific species. The symptoms are as follows:

1. The onset occurs 1 to 10 hours after ingestion of the fish.
2. The patient becomes acutely ill, with severe gastro-intestinal symptoms of nausea, vomiting, and diarrhea.
3. There is a distinct metallic taste in the mouth.
4. The skin is flushed and there is a tingling sensation and itching, which may last for a few days.
5. Cramps may occur in the extremities.
6. Hyperesthesia and paresthesia are commonly present. The paresthesia in which cold objects feel warm, is considered as one of the most significant symptoms.
7. There may be a weakness in the legs with temporary paralysis, and absent or reduced knee jerks.
8. There are muscular and joint aches present.
9. There is frequent, scalding urination, with albumin, granular casts, and mucus often present in the urine.
10. There may be nervousness, restlessness, and insomnia.

The severity of the symptoms vary with the individual. In view of the paucity of available data no correlation between the amount of fish eaten and the severity of the symptoms can be made. It is known, however, that not all individuals who eat poisonous fish will become ill. One attack does not impart immunity to Ciguatera, since individuals have experienced a second attack following closely a first attack. Usually the gastro-intestinal symptoms are of short duration, while the nervous symptoms may last for days or weeks with subsequent gradual recovery. To date no fatalities have been reported from this type of fish poisoning. (Tetrodon poisoning differs in this respect, see Yudkin 1944).

Mann (1938) stated that a diagnosis of fish poisoning can be made from the following data:

1. History of eating fish 1 to 10 hours before the onset of the illness.
2. A metallic taste in the mouth.
3. Paresthesia.
4. A prolonged convalescence.

Hurd (1945), however, indicated that the metallic taste in the mouth is of no diagnostic value, since in cases where repeated vomiting occurred due to causes other than fish poisoning, bile appearing in the mouth imparted a metallic taste. In view of the fact that severe muscle and joint pains are one of the chief complaints, it appears that more significance should be attached to these symptoms. The author believed that these symptoms are of greater diagnostic value than that of the metallic taste in the mouth. It is felt that the symptoms listed above distinguish Ciguatera, and make it a distinct entity from other types of animal, plant, mineral, or bacterial food poisoning.

Outbreaks of Poisoning

Numerous outbreaks of poisoning due to the ingestion of fish have been recorded in the areas where this poisoning exists. Only those will be considered which clinically resemble Ciguatera as described by Mann (1938) and O'Neill (1938). In the Caribbean area Walker (1922) recorded 13 outbreaks at St. Thomas, V. I., between the years 1918 and 1921, involving 70 individuals. Gregory (1925) reported 2 cases involving 5 men near St. Thomas. Costa Mandry (1928 and 1940) in Puerto Rico reported 2 outbreaks in 1928 involving 54 people and 1 in 1933 involving 39 individuals. O'Neill (1938) reported an outbreak near Culebra Island in 1938 in which 19 were poisoned. Gilman (1942) also reported one outbreak involving 10 individuals at Culebra Island. Schneck (1945) recorded an outbreak in which 14 individuals were poisoned in Puerto Rico. The U. S. Fish and Wildlife Service Laboratory in Mayaguez, Puerto Rico, has the following records of poisoning:

1. One case in 1944 involving 2 individuals.
2. One case in 1945 involving 11 people; and one in 1946 involving 40 people. (See Table 2).

Table 2.--Fish poisoning outbreaks in the Caribbean area recorded in the literature

	Authority	Date		Number of Outbreaks	Number of Individuals	Fish Responsible
1.	Gilman	May	1941	1	10	Sphyraena barracuda
2.	Schneck	Nov.	1944	1	15	1/
3.	O'Neill	Feb.	1928	1	19	Seriola falcata
4.	Gregory	Feb.	1925	1	2	Caranx latus
5.	Gregory	Feb.	1925	1	3	Caranx latus
6.	Walker 2/	Aug. Sept.	1918	-	34	Caranx sp.
7.	Walker	Oct.	1919	1	11	Caranx sp.
8.	Walker	July	1920	1	1	Caranx latus
9.	Walker	Aug.	1920	1	1	Caranx latus
10.	Walker	Feb.	1921	1	1	Seriola falcata
11.	Walker	June	1921	1	1	Seriola falcata
12.	Walker	July	1921	1	1	Seriola falcata
13.	Walker	Sept.	1921	1	1	Caranx sp.
14.	Walker	Sept.	1921	1	7	Scomberomorus cavalla
15.	Walker	Sept.	1921	1	1	Sphyraena barracuda
16.	Walker	Oct.	1921	1	8	Caranx sp.
17.	Walker	Oct.	1921	1	2	Sphyraena barracuda
18.	Walker	Nov.	1921	1	1	Caranx sp.
19.	U. S. F&WS	May	1944	1	2	Sphyraena barracuda
20.	U. S. F&WS	Feb.	1945	1	8	Scomberomorus cavalla
21.	U. S. F&WS	Aug.	1945	1	3	Sphyraena barracuda
22.	U. S. F&WS	April	1946	1	40	Caranx latus
23.	Costa Mandry	March	1928	1	25	Unknown
24.	Costa Mandry	May	1928	1	29	Unknown
25.	Costa Mandry	April	1933	1	39	Epinephelus morio

1/ Amberjack, king mackerel, and snappers were implicated in this outbreak.
2/ These records obtained by Walker from the Office of Sanitation at St. Thomas.

In the Pacific, Gatewood (1909) recorded an outbreak in Samoa in 1904 involving 20 individuals. Lee and Pang (1944) reported two outbreaks in Hawaii in 1944 involving 32 people. Vonfraenkel and Krick (1945) recorded one outbreak in the Marianas involving 30 people. Cohen, et al. (1946) reported two outbreaks in the Marianas involving 81 people. (See Table 3).

An accurate estimate of the number of cases of fish poisoning is impossible, because of the unreliable health statistics in many of the areas where the poisonings occur. (See Castillo 1945).

Table 3.--Fish poisoning outbreaks in the Pacific area recorded in the literature

Authority	Date	Number of Outbreaks	Number of Individuals	Fish Responsible
Lee and Pang	Nov. 1944	1	24	Black sea bass 1/
Lee and Pang	Nov. 1944	1	8	Red sea bass 2/
Vonfraenkel, et al.	Sept. 1944	1	30	Barracuda
Gatewood	1904	1	20	Lutjanus bohar
Cohen, et al.	May 1945	1	51	Ruvettus pretiosus 3/
Cohen, et al.	May 1945	1	30	Ruvettus pretiosus

1/ This is identified as Serranus fuscoguttatus from patients' description.
2/ This is identified as Variola louti .
3/ This was identified by Dr. L. P. Schultz from a description furnished by the investigators.

Theories Regarding Fish Poisoning

Numerous theories as to the causative agent or agents responsible for Ciguatera have evolved during the course of four centuries, but little has been done to either prove or disprove these theories by actual investigations. The most popular theories regarding the cause of fish poisoning are that it is either of endogenous origin or of bacterial origin.

Endogenous Origin

The former hypothesis suggests that the poisoning is due to a toxin which is formed either by the ovaries or testes of the fish during the spawning season, or is due to the fish feeding on certain material that does not harm the fish but renders the flesh toxic. A review of the literature revealed that most of these theories regarding the endogenous origin of the toxin are the result of analogy unsupported by any actual investigations.

An exception is made in regard to the puffers, on which much exacting biochemical work has been done in Japan. The ovaries of the female and to a lesser extent the spermaries of the male of these fish contain toxic substances during the breeding season. Also, the toxin has been found to be present in lesser concentrations in other tissues, particularly the viscera. The theory that fish which cause Ciguatera are toxic during spawning season has no factual basis for support. Nothing is known of the spawning habits of the fishes responsible for this poisoning. Gudger, an authority on the habits of the great barracuda, frankly admits nothing is known of the spawning habits of this fish (Gudger 1918). The only outbreak implicating a spawning fish is that reported by Gregory (1925) in which the causative fish was a female yellow jack carrying well developed roe. If spawning is the causative factor in Ciguatera, it might be expected that all sexually mature fish of a given species would be toxic. This has been shown to be not true.

In regard to fish becoming toxic from feeding on poisonous material, the natives of the islands of the Caribbean have implicated the following diverse sources:

1. Copper banks and copper contaminated water.
2. Manchineel berries from the manchineel tree (Hippomane mancinella).
3. Poisonous jelly fish or poisonous coral.
4. A "poisonous marine alga-cabriza."

Although one or another of these beliefs have been advanced by investigators as the causative agent of Ciguatera, the literature does not furnish any data to substantiate any of them. Most of these beliefs were held by the aboriginal Indians of the Caribbean, and have been either slightly modified or enlarged upon in the course of the last four centuries.

In a recent paper on the occurrence of fish poisoning in the Pacific, an attempt was made to make analogy of fish poisoning with mussel poisoning. Mussel poisoning has been ascribed to the dinoflagellate, Gonyolax catenella, which reputedly is eaten by the mussel, rendering it toxic. Since all the fish causing Ciguatera are carniverous pelagic species, it is hardly probable that they would feed on toxic plankton, jelly fish, coral, or berries. The possibility of fish becoming toxic from feeding on copper banks is also highly improbable, since all fish caught near copper banks are not toxic and conversely toxic fish are found in areas where there are no copper banks.

Bacterial Origin

Several investigators have suggested that the toxin is formed by bacteria present in the fish and derived from the fish's native habitat, or that it is produced by bacteria which have contaminated the fish in the process of handling. Most of the work done does not substantiate these theories. Very few bacteriological examinations have been made. Cohen, et al. (1946) reported that bacteriological examination of the fish responsible for poisoning implicated no organisms. Schneck (1945) attempted to isolate "causative organisms", with no success, from the feces of a dog which became ill after eating toxic fish. Lee and Pang (1945) in a fish poisoning case study stated, "Bacteriological examinations did not reveal any organisms on culture or direct smears." It is believed that these investigators referred to the common food poisoning bacteria, since it would have been highly improbable that no bacteria were present.

The only reliable bacteriological data concerning fish poisoning have been gathered by Costa Mandry (1928, 1933, 1940). In one outbreak involving 25 individuals in 5 families and causing the deaths of 2 dogs, some of the original lot of fish were examined bacteriologically. Bacillus proteus vulgaris, Escherichia coli, Alkaligenes fecalis, and Salmonella enteriditis were isolated.

In another outbreak 29 people out of a total of 45 who ate toxic fish became ill. No samples of the fish could be obtained, but some from a new lot from the same source were examined and found to contain Escherichia coli, Staphylococcus albus, Clostridium welchii, and Monilia psilosis. No feces could be obtained from any of the patients; however, blood samples from 10 of these cases were obtained for agglutination tests. Fifty percent showed agglutinins for Salmonella enteriditis or some organism of the Salmonella group.

In the third outbreak reported by Costa Mandry, 39 of the 42 people who had eaten fish became ill. It appeared that only those who ate the red grouper (Epinephelus morio) became ill. One sample of fried fish which caused poisoning was examined bacteriologically. A hemolytic yellow staphylococcus, Bacillus proteus, a Salmonella belonging to Group D with a somatic No. IX antigen, and an unidentified flagellar antigen were isolated. Toxicity tests on monkeys with the staphylococcus were negative. A water extract of the fish filtered through a fine Berkefeld filter fed to monkeys in 25, 30, and 35 ml amounts produced no symptoms.

Costa Mandry was of the opinion that bacteriological and epidemiological studies showed that most of the fish poisoning was due to contamination of the fish with food poisoning organisms during handling. However, the clinical symptoms of the outbreak he reported resemble Ciguatera rather than the symptoms of bacterial food poisoning. Mowbray (1916) cited a Dr. Georgaghan who indicated that there were two types of fish poisoning; one a ptomaine (bacterial food poisoning) and the other in the nature of a toxemia (Ciguatera). He further indicated that occasionally both types are combined or follow one another. Apparently the cases reported by Costa Mandry were of the combined form.

Seasonal Incidence of Poisoning

It is commonly believed that the poisoning is seasonal. Mowbray (1916) stated that barracuda, kingfish, jacks, and rockfish are most often toxic from May to October. O'Neill (1938) stated that near Puerto Rico the fish are most often toxic in November, December, and January. Walker (1922) noted that near St. Thomas, V. I., the horse-eyed jack (Caranx latus) is toxic most often in August and September. Gilman (1942), on the contrary, stated that natives of St. Thomas claim that the various species of jacks are most often toxic in May and June and the barracuda in May. A tabulation of 26 recorded outbreaks of poisoning in Puerto Rico and the Virgin Islands, shown in Table 4, reveals that poisoning is not nearly as seasonal as indicated in the literature.

TABLE 4.--A LIST, BY MONTHS OF OCCURRENCE, OF THE RECORDED FISH POISONING CASES IN THE CARIBBEAN AREA

Month	Number of Outbreaks	Species of fish responsible: Sphyraena barracuda	Seriola falcata	Scomberomorus cavalla	Caranx spp.	Epinephelus morio	Unknown
January	0	0	0	0	0	0	0
February	5	0	2	1	2	0	0
March	1	0	0	0	0	0	1
April	2	0	0	0	1	1	0
May	3	2	0	0	0	0	1
June	1	0	1	0	0	0	0
July	2	0	1	0	1	0	0
August	3	1	0	0	2	0	0
September	4	1	0	1	2	0	0
October	3	1	0	0	1	0	0
November	2	0	0	0	1	0	1*
December	0	0	0	0	0	0	0

* THIS CASE INVOLVING 15 INDIVIDUALS WAS REPORTED CAUSED BY AN ASSORTMENT OF FISH CONSISTING OF AMBERJACK, KING MACKEREL, AND SNAPPER.

Size of Fish as a Factor in Poisoning

Numerous investigators believed that the size of the fish is an important factor in poisoning. Large fish are supposedly more toxic than small fish of the same species. Some authorities believe that small fish are never toxic. There is little dependable information regarding the role of the size of the fish in poisoning. Most of the outbreaks recorded in the literature, however, have been due to large fish. Walker (1922) reported one outbreak caused by a small (3 lb.) barracuda.

Nature of the Poison

The poisonous substance responsible for fish poisoning has been described as an alkaloid (a toxalbumin), however, a thorough search of the literature does not reveal any data to uphold this view. The available data regarding the causal agent of Ciguatera does indicate that the toxic substance is very thermostable. In the outbreak reported by O'Neill (1938) the flesh of the fish proved to be toxic after having been broiled for 20 minutes and then baked in an oven for $2\frac{1}{2}$ hours.

It has been suggested that there is a correlation between toxicity and the length of time between catching and cooking of the fish. Here again, no relevant data has been presented to uphold this view. In the outbreaks reported by Gregory (1925), the yellow jacks responsible for the poisonings were cooked immediately after they were caught.

Methods of Determining Toxicity

The natives of the West Indies have numerous methods for determining whether or not a fish is poisonous, but none seems to be reliable. The people of tropical and subtropical areas, where fish poisoning is prevalent, often have to depend on fish as a source of food supply. Because poisonous fish cannot be recognized by observation or by any infallible test, it is apparent that a determination of the source of poisoning and methods of prevention would solve a vital problem. Therefore, an investigation of Ciguatera was conducted by the U. S. Fish and Wildlife Service in the areas near Puerto Rico and the Virgin Islands in 1945.

PART II--FISH AND WILDLIFE SERVICE INVESTIGATION OF THE PROBLEM

Since no source of poisonous fish could be found in Puerto Rico during the early phase of the investigations, a field laboratory was established at the U. S. Naval Hospital at Bourne Field, in Charlotte Amalie, St. Thomas, V. I. Preliminary work was conducted in this temporary laboratory until arrangements were made to insure an adequate supply of fish; later the work was continued at the Fishery Research Laboratory at Mayaguez, P. R.

Source of Fish

The fish used were from those species considered poisonous by the French fishermen off St. Thomas and by the native fishermen of Jost Van Dyke Island. The fish obtained from the French fishermen were caught west-southwest of St. Thomas in the general vicinity of Sail Rock which is located in a supposedly poisonous area. The fish obtained from the fishermen of Jost Van Dyke Island were caught in the waters between St. Thomas and St. John. Occasional specimens of fish were obtained from sports fishermen, most of which were caught near St. Thomas. Most of these fish were out of water on the average of eight hours without refrigeration before they were landed. During the course of work in St. Thomas, the fish were examined, whenever possible, immediately on their arrival at the laboratory. Otherwise, they were refrigerated or frozen and held until they could be examined. When the study was conducted in Mayaguez, the source of supply of fish was from the St. Thomas area. The fish were frozen there, then shipped in the frozen state to Mayaguez, and held frozen until they could be examined. The effect of freezing on the toxicity of fish is not known, however, this was the only way of insuring an adequate supply of fish for the tests.

Laboratory Procedures

General

On arrival at the laboratory, the fish were identified as to genus and species. Their length, width, and weight were recorded. The size, condition, and weight of the gonads and the general condition of the viscera were noted. The contents of the stomach were then examined, and a macroscopic examination for the presence of endoparasites and ectoparasites was made.

Bacteriological

A quadrangular section of the skin was removed aspetically from the flesh of the fish. With sterile instruments a 20 gram sample of muscle was excised and weighed into a sterile, tared petri dish. This fish flesh was then transferred into a sterile Waring Blendor, 180 mls of sterile buffer solution 1/were added, and the mixture was thoroughly macerated. Samples of this mixture were used for all subsequent bacteriological tests.

Tryptone glucose extract agar was employed for plate counts, and standard lactose broth was used for determining the M.P.N. of coliforms. S. S. agar, MacConkey's agar, and bismuth sulfite agar were used for determining the presence of enteric pathogens. Tetrathionate broth was used as an enrichment medium. The Most Probable Number method was used for determining the number of anaerobes, employing Difco anaerobe medium. All samples were incubated at 37° C. Plate counts and the M.P.N. determinations of anaerobes were made at the end of 40 hours incubation. The M.P.N. of coliforms was made according to the procedures outlined in Standard Methods for the Examination of Water and Sewage (1936). Representative colonies that appeared on tryptone glucose extract agar were transferred to agar slants for further identification. Coliform colonies found on Levine's eosine methylene blue agar were also transferred for further identification. All colonies were purified on tryptone glucose extract agar plates previous to identification. Smears were made from the anaerobe medium, stained by the Gram method, and examined for the presence of organisms resembling the genus Clostridium.

Toxicological

A modification of the method used by Macht and Spencer (1941) for testing the toxicity of fish muscle was employed. A 50 gram sample of muscle was removed in the manner identical to that employed in obtaining the sample for bacteriological examination. This was placed in a chemically clean Waring Blendor, 100 ml of sterile 0.8 percent saline were added, and the mixture was thoroughly macerated. A portion of the mixture was placed in a glass tube and centrifuged until a clear liquid supernate was obtained. Gonad tissue was prepared in a similar manner. The sample weight of the gonad tissue varied, however, the proportion of saline used was the same as that used in the preparation of the muscle tissue extract. The clear supernate was used for the tests.

Three white mice, each weighing between 20 and 25 g, was each injected intraperitoneally with 1 ml of the saline extract made from the raw tissue. These mice were then observed closely for 1 hour and after that at periodic intervals. In those cases where all injected mice died, the saline extract of the raw sample was heated to 80° C. for ½ hour (an arbitrary temperature and time) and another series of 3 mice was injected with 1 ml of the heated extract. In those cases where the mice exhibited a positive reponse to the intraperitoneal injection of the extract, the raw tissue was fed orally to another group of animals. For the oral feeding tests, three mice that had been fasted for 24 hours prior were used. No necropsies were performed.

1/ The method for preparation of the buffer solution is as follows: Dissolve 34 g of KH_2PO_4 in approximately 500 ml of distilled water. Adjust to pH 7.2 with 1 N NaOH. Dilute to 1 liter. Add 1.25 ml of this stock buffer solution to each liter of distilled water.

Fish Examined

A total of 46 samples of fish were examined in the course of this study (Table 5): of these 36 were the yellow jack (Caranx bartholomaei); 2 each of the great barracuda (Sphyraena barracuda) and the cero (Scomberomorus regalis); and 1 each of the hogfish (Lachnolaimus maximus), the horse-eyed jack (Caranx latus), the amberjack (Seriola falcata), and the yellowtail snapper (Ocyurus chrysurus). One sample of barracuda examined was from a lot which had been responsible for the poisoning of three individuals. The raw muscle of all 46 samples was examined toxicologically. However, it was possible to examine the gonads of only 16 of the samples, since in the remaining 30 samples either the gonads were too small to examine or the fish had been eviscerated and the material discarded. Twenty-nine samples of fish flesh were examined bacteriologically, of which 12 were examined for anaerobic bacteria in addition to the other routine bacterial examinations.

Table 5.--Species and Sex of Fish Examined

Species	Male	Female	Unknown*
Scomberomorus regalis	1	1	0
Sphyraena barracuda	0	1	1
Lachnolaimus maximus	0	0	1
Caranx crysos	2	0	0
Caranx latus	0	0	1
Caranx bartholomaei	19	16	1
Seriola falcata	0	0	1
Ocyurus chrysurus	0	0	1

*Identification as to sex not possible since these fish had been either gutted or a small portion of flesh was received for examination.

The viscera of all fish appeared normal on examination. No parasites were observed. The stomachs of all fish were empty, with the exception of one male cero, which contained two small partially digested fish so decomposed that they could not be identified.

Results of Bacteriological Examinations

The plate counts ranged from 10 to 28,600 bacteria per gram of muscle. Coliform organisms were present in 7 samples; 6 samples contained a M.P.N. of 10, and 1 sample a M.P.N. of 16,000 coliform bacteria per gram of fish muscle. The M.P.N. of anaerobes ranged from 1.1 to 160 bacteria per gram of muscle (Table 6).

Plates of S.S. agar, MacConkey's agar, and bismuth sulfite agar, were streaked directly and from tetrathionate broth cultures but failed to show any colonies resembling the Salmonella, Eberthella, or Shigella groups.

No enteric pathogens were encountered on any of the differential media; however, there was isolated from a female yellow jack an organism having the cultural and biochemical characteristics of Salmonella enteriditis. A typical aerogenes colony was isolated on Levine's eosin methylene blue agar which had been seeded from a tube of lactose broth. This colony was transferred to a nutrient agar slant. The culture was purified by streaking on a tryptone glucose agar. Two morphologically distinct colonies were then obtained which were designated C9S and C9R. On further cultural and biochemical studies it was found that culture C9S was typical Aerobacter oxytocum and culture C9R a typical Salmonella enteriditis.

No colonies resembling Staphylococci were present on tryptone glucose extract agar plates, and smears made from anaerobe medium failed to reveal any organisms resembling the Clostridium group.

The bacteria isolated comprising 58 specimens fell into 10 genera and 27 species which were distributed as follows:

1. Micrococcus - 13 species.
2. Flavobacterium - 3 species.
3. Escherichia - 3 species.
4. Aerobacter - 2 species.
5. Alcaligenes, Proteus, Pseudomonas, Salmonella, Bacillus, and Sarcina - 1 species of each.

It is interesting to note that only 11 of the 27 species are reported as being of marine origin. Of these were 4 specimens of Micrococcus perflavus, 2 of Micrococcus varians, 2 of Alcaligenes viscosus, and 1 each of Flavobacterium turcosum and Pseudomonas non-liquefaciens. Table 6 shows the species of bacteria isolated from each fish sample.

TABLE 6.--RESULTS OF BACTERIOLOGICAL EXAMINATION OF VARIOUS SAMPLES OF FISH MUSCLE

SAMPLE NUMBER	SPECIES OF FISH	BACTERIA PER GRAM OF MUSCLE	M.P.N. OF COLIFORM BACTERIA PER GRAM OF MUSCLE	M.P.N. OF ANAEROBIC BACTERIA PER GRAM OF MUSCLE	SPECIES OF BACTERIA ISOLATED
1	SCOMBEROMORUS REGALIS	700	0	-	(2)
2	DO	9,100	0	-	(2)
3	CARANX CRYSOS	- 1/	0	-	MICROCOCCUS PERFLAVUS
4	DO	3,050	0	-	MICROCOCCUS PERFLAVUS, SARCINA VENTRICULI, ESCHERICHIA COLI (ATYPICAL)
5	LACHNOLAIMUS MAXIMUS	3,100	0	-	MICROCOCCUS CANDIDUS, M. ROSEUS, FLAVOBACTERIUM BREVE
6	SPHYRAENA BARRACUDA	- 1/	16,000	-	ESCHERICHIA COLI
7	DO	190	0	-	MICROCOCCUS RHODOCHROUS, M. LUTEUS
8	SERIOLA FALCATA	300	0	-	(2)
9	CARANX LATUS	1,400	0	-	(2)
10	OCYURUS CHRYSURUS	860	0	-	(2)
11	CARANX BARTHOLOMAEI	1,360	0	-	MICROCOCCUS EPIDERMIDIS
12	DO	4,700	10	-	MICROCOCCUS LUTEUS, M. CANDIDUS, SALMONELLA ENTERIDITIS, AEROBACTER OXYTOCUM
13	DO	28,600	10	-	MICROCOCCUS AURANTIACUS, M. VARIANS, ESCHERICHIA COLI (ATYPICAL)
14	DO	11,200	10	-	FLAVOBACTERIUM TURCOSUM, PROTEUS SP., ESCHERICHIA COMMUNIOR
15	DO	12,400	10	-	MICROCOCCUS VARIANS, M. CANDIDUS, AEROBACTER CLOACAE
16	DO	14,300	10	-	MICROCOCCUS PERFLAVUS, M. SACCATUS, AEROBACTER CLOACAE
17	DO	6,200	10	-	BACILLUS CIRCULANS, AEROBACTER CLOCACAE
18	DO	30	0	17	MICROCOCCUS EPIDERMIDIS, FLAVOBACTERIUM ESTEROAROMATICUM (ATYPICAL)
19	DO	110	0	54	MICROCOCCUS EPIDERMIDIS
20	DO	30	0	14	MICROCOCCUS CANDIDUS
21	DO	10	0	1.1	MICROCOCCUS EPIDERMIDIS
22	DO	310	0	160	SARCINA VENTRICULI, MICROCOCCUS SP.
23	DO	370	0	3.3	MICROCOCCUS PERFLAVUS, MICROCOCCUS SP., MICROCOCCUS SP.
24	DO	20	0	1.3	ALCALIGENES VISCOSUS, MICROCOCCUS SP.
25	DO	30	0	4.9	MICROCOCCUS SP., M. SP.
26	DO	1,570	0	160	PSEUDOMONAS NON-LIQUEFACIENS, MICROCOCCUS SACCATUS
27	DO	40	0	35	MICROCOCCUS SACCATUS, M. CEREUS, ALCALIGENES VISCOSUS
28	DO	20	0	54	MICROCOCCUS FLAVUS, M. PERFLAVUS
29	DO	280	0	160	MICROCOCCUS CREMORIS VISCOSI, M. LETEUS, FLAVOBACTERIUM SP.

1/ THESE SAMPLES CONTAINED AN EXCESSIVE NUMBER OF BACTERIA. NO EXACT COUNT WAS OBTAINED SINCE A SUFFICIENTLY HIGH DILUTION OF THE SAMPLE WAS NOT MADE TO ALLOW ACCURATE COUNTING OF THE PLATE CULTURES.

2/ NO ISOLATIONS MADE.

Results of Toxicological Examinations

Fifteen out of 46 extracts of raw muscle elicited some response in mice. Two of these samples killed all 3 mice in the series. Twelve of the 16 gonad extract samples also elicited a reaction in mice, while 2 of these samples killed all 3 of the mice used in the series. From the 16 samples in which both gonad and muscle were examined, the following reactions were obtained (Table 7):

1. In 5 samples, both muscle and gonad extract elicited a response.
2. In 7 samples only the gonad extract elicited a response.
3. In 1 sample only the muscle extract elicited a response.
4. In 3 samples neither the gonad nor the muscle extract elicited a response.

In all instances where a fatal response was obtained by intraperitoneal injection of the saline extract of the raw sample, there resulted either no response or a non-lethal response from injection of the heated saline extract. Furthermore, no response was obtained as a result of feeding mice the raw tissue.

Various types of reactions resulted from the injection of the saline extracts. A positive response was characterized by the following general reactions: On injection the mouse would arch its back and would show symptoms of cramps in the abdomen; often this was the only response and lasted for only a few minutes. There followed rapid, shallow breathing usually associated with this initial reaction. These symptoms would disappear in $\frac{1}{2}$ to 3 hours; however, those animals showing severe reaction eventually died. In the instances where death occurred, it occurred 5 to 10 hours after the injection.

In view of the fact that none of the raw tissue from which the saline extracts were made caused deaths on feeding, the fatal reactions cannot be attributed to toxicity of the raw muscle or gonad. A possible explanation for the responses is that the blood and sera of certain species of fish is known to be toxic to most laboratory animals on parenteral administration. For an excellent review of this phenomenon, the reader is referred to Phisalix (1922) or Calmette (1908).

TABLE 7.--RESULTS OF THE TOXICOLOGICAL EXAMINATIONS OF MUSCLE AND GONAD EXTRACTS

SPECIES	NUMBER OF FISH EXAMINED	SEX	REACTION OF MICE TO INTRAPERITONEAL INJECTION	
			MUSCLE EXTRACT	GONAD EXTRACT
CARANX BARTHOLOMAEI	5	F	+	+ 1/
DO	5	F	0	+ 2/
DO	1	F	+	0
DO	1	F	0	0
DO	4	F	0	-
DO	10	M	0	-
DO	1	M	0	+
DO	8	M	+ 3/	-
DO	1	?	0	0
SCOMBEROMORUS REGALIS	1	F	0	0
DO	1	M	0	0
SPHYRAENA BARRACUDA	1	F	0	+
DO	1	?	+ 4/	-
CARANX CRYSOS	2	M	0	-
LACHNOLAIMUS MAXIMUS	1	?	0	-
SERIOLA FALCATA	1	?	0	-
OCYURUS CHRYSURUS	1	?	0	-
CARANX LATUS	1	?	0	-

LEGEND: +, REACTION; 0, NO REACTION; -, NO SAMPLE.

1/ ONE SERIES OF 3 MICE WAS KILLED BY RAW GONAD EXTRACT IN 6½ HOURS. HEATED EXTRACT (½ HOUR AT 80° C.) ELICITED NO REACTION. RAW GONAD TISSUE ELICITED NO REACTION ON FEEDING.

2/ ONE SERIES OF 3 MICE WAS KILLED BY RAW GONAD EXTRACT IN 10 HOURS. HEATED EXTRACT (½ HOUR AT 80° C.) ELICITED NO REACTION. RAW GONAD TISSUE ELICITED NO REACTION ON FEEDING.

3/ ONE SERIES OF 3 MICE DIED FROM RAW MUSCLE EXTRACT IN 5 HOURS. HEATED EXTRACT (½ HOUR AT 80° C.) ELICITED SLIGHT REACTION. ANIMALS FULLY RECOVERED IN 18 HOURS. RAW TISSUE FED ELICITED NO REACTION.

4/ ONE SERIES OF 3 MICE DIED IN 8¼ HOURS FROM RAW MUSCLE EXTRACT. ONE MOUSE RECEIVING 1 ML OF HEATED EXTRACT DIED IN 14 HOURS. TWO MICE RECEIVING 0.5 ML OF THE SAME EXTRACT SHOWED MARKED REACTION BUT RECOVERED. RAW TISSUE ELICITED NO REACTION ON FEEDING.

Case Report and Results of Bacteriological and Toxicological Study of an Outbreak of Barracuda Poisoning in St. Thomas, V. I.

In the course of the investigation at St. Thomas, one case of barracuda fish poisoning was observed. The data regarding the outbreak and the bacteriological results are described as follows:

The outbreak was brought to the attention of the laboratory by Dr. D. H. Snyder of the Municipal Hospital, Charlotte Amalie, St. Thomas, V. I., who diagnosed it as a case of fish poisoning. The fish, a great barracuda, approximately 4½ feet long, had been caught in the harbor of St. Thomas near the submarine base in the late afternoon of August 6, 1945. The fish was gutted immediately after it was caught. The flesh was knife-scored (diagonal incision made approximately 1 inch apart on both sides of the fish) and then sun-dried. A portion of this fish was given to some friends; however, these individuals could not be located to determine whether or not they had been

poisoned. At 11:00 a.m., August 8, three natives, two adult males and one adult female ate some of the fish. Early the next evening, they became ill with symptoms of nausea, vomiting, and diarrhea. One male apparently recovered the next day. In the late afternoon of the 9th, Dr. Snyder was called in to see the other two patients, both of whom were quite ill. He treated them symptomatically. A specimen of the feces was obtained from one of the male patients and a sample of the barracuda was obtained from a raw piece of flesh that had been thrown into a chicken run. The female patient was able to get up on the morning of August 10 and the male on August 11. All traces of the patients were lost after August 11 since they promptly disappeared. It is impossible, therefore, to determine whether or not they had any of the residual symptoms associated with fish poisoning.

Bacteriological examination of the fecal sample failed to show any organisms of the enteric group of pathogens. The barracuda sample was not suitable for bacteriological examination since it had been found in a chicken run, nevertheless it was examined. Plating the material on differential agar media showed negative for enteric pathogens. The sample contained an excessive number of bacteria. Tryptone glucose extract agar plates were overgrown and impossible to count. The dilution of sample used was not great enough to permit an accurate bacterial count. The flesh contained a minimum probable number of 16,000 Coliform bacteria per gram. However, none of these particular data is regarded as significant since the sample available was exposed to considerable contamination prior to examination. For the toxicological tests a saline extract of the raw muscle was prepared in accordance with the procedure described in the foregoing discussion. One milliliter of this extract was injected intraperitoneally in each of a series of three mice. The results are shown in detail as follows:

Mouse Number	Amount of Inoculum	Time of Inoculation	Reaction[1/]
7	1.0 ml	10:11 a.m.	Dead at 5:45 p.m.
8	1.0 ml	10:16 a.m.	Dead at 3:15 p.m.
9	1.0 ml	10:16 a.m.	Dead at 6:30 p.m.

1/ Immediately after being injected the mice hunched their bodies and appeared to have cramps in their abdomens. They dragged their hind quarters trying to walk. Ten minutes later they appeared normal. Half an hour later, however, they started to develop a rocking, rolling gait, resembling a drunken person with a progressive paralysis of the hind quarters. This paralysis became worse until death ensued.

Some of the extract was then heated at 80° C. for ½ hour and another series of 3 mice was each injected intraperitoneally with 1 ml of the heated extract.

Mouse Number	Amount of Inoculum	Time of Inoculation	Reaction
10	1 ml	6:49 p.m.	1/
11	1 ml	6:43 p.m.	1/
12	1 ml	6:46 p.m.	2/

1/ Mice reacted with symptoms similar to those resulting from the injection of the unheated extract; however, they recovered completely by 8:00 a.m. the following morning.

2/ Mouse No. 12 had symptoms similar to those resulting from the injection of unheated extract and died at 9:10 a.m. the following morning.

Ten grams of the raw flesh were fed each of three mice that had been fasted for 24 hours. The mice showed no ill effects. No definite conclusions regarding the nature of the poisoning agent can be made from the above data.

Discussion

An extensive review of the literature on fish poisoning discloses that very little is definitely known regarding this phenomenon except the symptomology, a few of the species of fish involved, and the localities where the poisoning is prevalent. Some investigators have placed into print native folklore under the guise of scientific investigation. Numerous references are made as to the nature of the "toxic agent," seasonal incidence of poisoning, and methods for distinguishing poisonous from non-poisonous fish, without an iota of research data to support them. It is hoped that this paper will dispel some of the erroneous ideas, posed as scientific facts, regarding fish poisoning.

The present investigations have done little to enlarge our knowledge of fish poisoning. It was found fish poisoning is not a seasonal phenomenon.

No progress concerning the cause or the causative agent of fish poisoning can be made unless some actual research work is done. An active research program is necessary to prove or to disprove the various theories regarding fish poisoning. These investigations should consist of the following studies:

1. Studies of the life history and ecology of the various species of fish responsible for poisoning.
2. Feeding experiment using more suitable test animals.
3. Extensive bacterial and chemical tests on samples of fish which are found to be toxic to laboratory animals in order to try to find the causative agent of the disease.
4. Collection of epidemiological data on all so-called "fish poisoning cases," to determine whether or not they are the result of true "fish poisoning," food poisoning, or a combination of the two.

Summary

1. Ciguatera has been reported as being distributed rather extensively in fairly well scattered areas in tropical and subtropical waters of the Atlantic and Pacific Oceans.

2. It is limited to a few species of fish. However, not all fish of the same species caught at the same time in the same area are toxic.

3. The poisoning is not seasonal.

4. The toxin appears to be thermostable.

5. The findings of this investigation as well as those of Costa Mandry (1933 and 1940) indicate that some of the fish may be infected with enteric pathogens.

6. A more intensive active research program is needed to determine the cause and/or causative agent of Ciguatera.

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